

CLAIMS

1. Method which allows cells to acquire the capacity to produce a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises the following steps:

a) transforming said cells by at least one introduction of a missense mutation in a target codon of a gene encoding a protein required for the growth of said cells, said protein synthesized from the gene thus mutated no longer being functional;

b) where appropriate, culturing the cells obtained in step a) in a culture medium containing a nutrient compensating for the loss of functionality of said protein thus mutated; and

c) culturing the cells obtained in step a) or b) in a culture medium containing the amino acid encoded by said target codon.

2. Method according to claim 1, characterized in that the culture medium of step c) does not contain the nutrient required by the loss of functionality of said mutated protein.

3. Method according to either of claims 1 and 2, characterized in that step c) for culturing said cells comprises a series of cultures of said cells in a culture medium containing the amino acid encoded by said target codon, each of said cultures of the series being prepared as far as obtaining the stationary growth phase and followed by washing of the cells obtained, the number of cultures of the series being sufficient to allow the selection of mutations which increase the suppression of said missense mutation of said mutated gene.

4. Method according to one of claims 1 to 3, characterized in that the missense mutation is chosen from missense mutations which spontaneously reverse

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only at very low frequency, of the order of one organism from at least 10^{15} .

5. Method according to one of claims 1 to 4, characterized in that the missense mutation transforms a target codon of a gene encoding a protein required for the growth of said cell, into a codon which, in comparison with the target codon, exhibits a change of at least two bases, preferably three bases.

6. Method according to one of claims 1 to 5, characterized in that the target codon encodes an amino acid which has a small steric volume.

7. Method according to one of claims 1 to 6, characterized in that the target codon encodes an amphiphilic amino acid.

8. Method according to one of claims 1 to 7, characterized in that the target codon encodes an amino acid which has a steric volume smaller than or substantially equal to the steric volume of the amino acid encoded by the missense mutation.

9. Method according to one of claims 5 to 8, characterized in that the target codon encodes cysteine.

10. Method according to one of claims 5 to 9, characterized in that the amino acid encoded by the missense mutation is valine or isoleucine.

11. Method according to one of claims 1 to 10, characterized in that step a) for transforming said cells is carried out using a vector comprising a sequence of said gene encoding a protein required for the growth of said cells, including said missense mutation.

12. Method according to claim 11, characterized in that said vector is a plasmid vector.

13. Method for selecting cells capable of producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises steps a), where appropriate b), and c) of a method according to one of claims 1 to 12, and selecting the cells capable of growing in step c).

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c) isolating said protein comprising at least one unconventional amino acid from the culture supernatant and/or from the cell pellet obtained in step b).

5 26. Process according to claim 25, characterized in that said culture medium of step b) which allows the growth of said cell contains said unconventional amino acid or a precursor thereof.

10 27. Process according to claim 25, characterized in that said unconventional amino acid is synthesized by said cell.

28. Process according to claim 27, characterized in that the synthesis of said unconventional amino acid is increased by genetic modification of said cell.

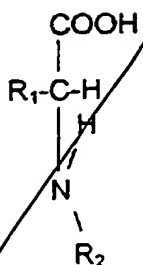
15 29. Process according to one of claims 25 to 28, characterized in that said cell is auxotrophic for the amino acid encoded by said target codon.

20 30. Process according to one of claims 25 to 29, characterized in that said cell comprises a homologous or heterologous gene of interest the coding sequence of which includes at least one target codon.

25 31. Process according to claim 30, characterized in that step b) comprises the compounds required for inducing the synthesis of the protein encoded by said gene of interest.

30 32. Process according to claim 30 or 31, characterized in that the biological activity of the protein encoded by said gene of interest is at least partially conserved after the incorporation of said unconventional amino acid at the level of the target codon of said gene of interest.

35 33. Process according to one of claims 25 to 32, characterized in that the unconventional amino acid is chosen from unconventional amino acids of formula I of configuration L



(I)

in which:

R₁ or R₂ represents radicals containing a functional group capable of reacting selectively.

34. Process according to claim 33, characterized in that the functional group is chosen from aldehyde, ketone, ethenyl, ethynyl and nitrile groups.

35. Process according to one of claims 25 to 34, for protein functionalization.

36. Protein purification process, characterized in that it comprises the following steps:

a) incorporating into the amino acid sequence of said protein an unconventional amino acid containing a functional group capable of reacting selectively, using a process according to one of claims 25 to 35;

b) bringing the solution containing the protein obtained in step a) into contact with a support comprising a compound capable of reacting specifically with said functional group and of attaching specifically said protein; and

c) isolating said protein attached to the support.

37. Process for attaching a protein to a chemical or biochemical compound, characterized in that it comprises the following steps:

a) incorporating into the amino acid sequence of said protein, by a process according to one of claims 25 to 35, an unconventional amino acid containing a functional group capable of reacting selectively;

b) bringing the protein obtained in step a) into contact with said chemical or biochemical compound comprising a group capable of reacting

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specifically with said functional group in a medium allowing the reaction.

38. Process according to claim 37, characterized in that said chemical or biochemical compound is, itself, attached to a solid support or is a constituent compound of a solid support.

39. Process according to claim 37 for preparing a protein complex.

40. Process according to claim 39, characterized in that the attached protein or the chemical or biochemical compound is chosen from therapeutic, cosmetic or diagnostic compounds.

41. Process according to claim 39 or 40, characterized in that the chemical or biochemical compound is chosen from compounds capable of modifying the biological activity of the attached protein.

42. Process according to claim 39 or 40, characterized in that the chemical or biochemical compound is chosen from compounds the biological activity of which can be modified by the attached protein.

43. Process according to one of claims 39 to 42, characterized in that the chemical or biochemical compound is chosen from compounds comprising a protein, a polynucleotide, a fatty acid, a sugar or a natural or synthetic polymer.

44. Protein obtained using a process according to one of claims 25 to 36.

45. Protein according to claim 44, characterized in that it is a recombinant protein.

46. Protein complex obtained using a process according to one of claims 39 to 43.

47. Use of a protein according to claim 44 or 45, or of a protein complex according to claim 46, as a diagnostic reagent.

48. ~~Diagnostic process, characterized in that it uses a protein according to claim 44 or 45, or a protein complex according to claim 46.~~